THE EFFECT OF HUMIDITY, LOCATION, SURFACE FINISH AND SEPARATOR THICKNESS ON THE DRY HEAT DESTRUCTION OF BACILLUS SUBTILIS VAR. NIGER SPORES LOCATED BETWEEN MATED SURFACES

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INTRODUCTION

This is a continuation of studies reported in Progress Reports #1 and #2. Report #1 described the mated surface experimental system and summarized the preliminary experiments which were performed. These indicated that relative humidity before and during treatment and position within the mated surface were the most important parameters affecting microbial destruction. Other variables of interest were the surface finish of the mated surface and the presence or absence of separators between the surfaces.

A series of experiments was conducted to explore the effects of the parameters listed above. In addition an open system was prepared and tested to provide experimental data comparable to that for the mated surface system. Preliminary data were reported in Progress Report #2. The present report presents the analyzed results of experiments performed after November, 1968.

OBJECTIVE

The objective of this project is to measure the thermal death parameters of Bacillus subtilis var. niger spores in a mated surface system and to determine the effect of certain variations of the environment and surfaces on thermal destruction.

EXPERIMENTAL PROCEDURE

Details of the experimental procedure are contained in Reports 1 and 2 (December, 1968 and June, 1969). A sketch of the treatment apparatus with mated surface package in place is shown in Figure 2.1. The effort in this project during the reporting period was used to carry out the

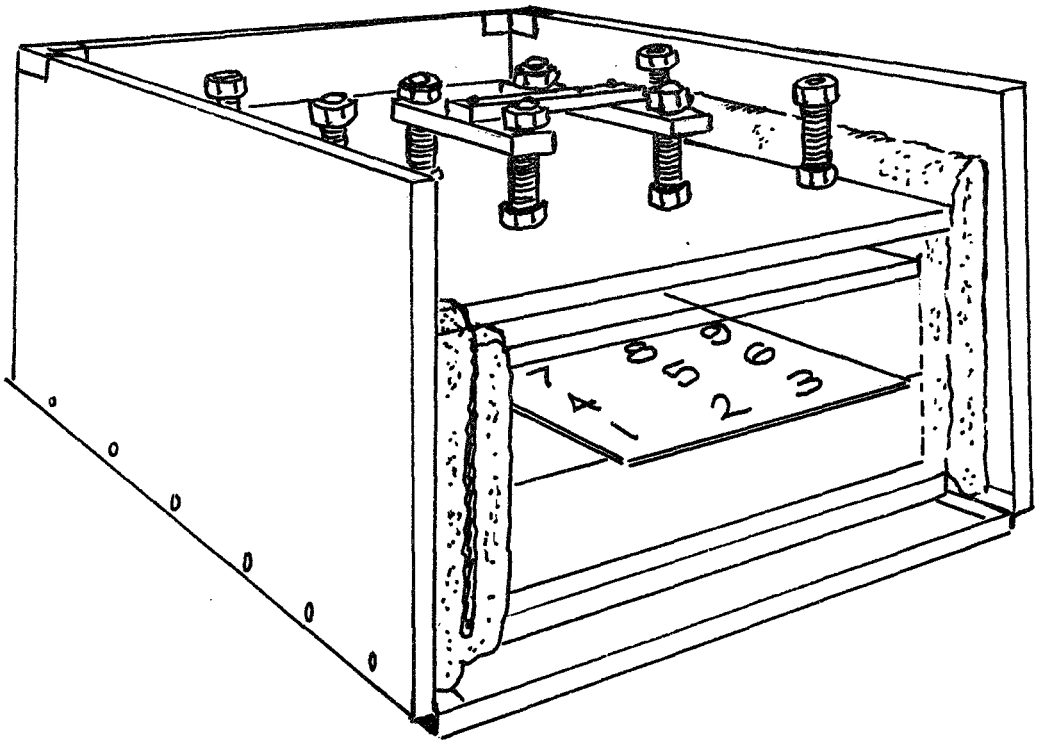

Figure 2.1 - The heat block used for treating mated surface packages is shown with a package in place. The numbers represent the location of the spore deposits on the 6" x 8" mated surface.

following new experiments:

1. Mated System
   a. Measurement of microbial destruction parameters of spores after conditioning in a clean room .
   b. The first of a series of tests to compare the thermal resistance of Bacillus subtilis var. niger with the thermal resistance of spores isolated by Jet Propulsion Laboratory from the Mariner Spacecraft.
   c. A test of the effect of the concentration of spores in a mated surface area on the D-value.
2. Open System
   a. Experiments at high and low conditioning and treatment humidities were repeated with the time for movement from the conditioning to the treatment environment reduced from four minutes to approximately twenty seconds.
   b. An experiment with spore conditioning and treatment in a clean room provides data at intermediate relative humidity conditions.
3. Development of detailed data analysis computer programs.
   a. Analysis of variance. The analysis of variance was carried out by the statistical services group of the Biometry Department of the School of Public Health, University of Minnesota. The model used to analyze all data was a combination of factorial and split-plot approaches. In both the mated surface and the oven systems each group of samples which received a given time-temperature treatment combination was considered to be a whole plot. The individual samples within the time-treatment combination (i.e.the positions within the mated surface package) were the sub-plots. A factorial analysis of the whole plot allowed separation of the effects of day of treatment, conditioning humidity, treatment humidity, surface finish, etc. as appropriate to a given experiment. The split-plot analysis determined whether there were variations among positions; such variations would be expected in the mated

system but not in the open system. Since analysis of variance assumes that the variance of individual samples is a constant, it was necessary to modify the data so that the assumption would hold. The analysis therefore, was carried out on the logarithms of the data values. Since all data values were included, even those which were zero, the actual modifying function used was the logarithm of datum value +1.0. The modification introduces a small error in the data, but it is not significant above an estimated value of twenty spores where it produces a 5% error. The modification allows the direct computer analysis of the data.

b. Linear regression. The linear regression was carried out by a computer program developed specifically for the analysis of microbiological data. The program performs the following tasks:
   1. Lists the raw data
   2. Computes and lists the logarithm of datum value +1.0
   3. Computes and lists the geometric mean, standard error and the 95% confidence interval for replicate values for each exposure time
   4. Performs a least-squares linear regression analysis on the data
   5. Calculates and prints the D-values, the y-intercept and their 95% confidence intervals
   6. Calculates separate regression lines and D-values for each column (e.g. position)

RESULTS AND DISCUSSION

The results are summarized experiment-by-experiment in this section. Where applicable, an analysis of variance was used to determine statistical significance. The D-values and y-intercepts of the regression lines, the 95% confidence interval for each, and the intercept ratio (IR) where IR equals $\log y_o / \log N_o$ are recorded in the data table. The IR is a gross measure of the shape of the survivor curve during the initial

portion of the heat treatment (i.e. the portion between t=o and the shortest treatment time.) If the IR is greater than 1.0 there may be a trend for the D-value to decrease with increasing heating times; that is for the curve to be concave downwards, the curve may be thought to have a shoulder. If the IR is less than 1.0 the initial portion of the curve is concave upwards. The logarithms were used to calculate the IR since the intercept of the regression line and the value of N_o are both calculated using the logarithms of the data. If further analysis of the IR is desired at a later time it will be less complex than the analysis of a statistic which was composed of functions of other statistics.

The analysis of variance was performed using all recovered data. In all cases where the analysis was performed, six positions from the mated surface plate were included. To make the present report manageable, however, only the results for positions 1 and 5 have been reported in the tables.

Experiment DD8320

The experiment evaluated the effect of four treatment humidities on the D-value of spores in the mated surface system. All samples were conditioned at 40% relative humidity and 23°C in a clean room; the treatment humidities were .36%, .66%, .79%, and 1.1% at 125°C.

The treatment humidities were obtained by placing the treatment apparatus in a walk-in refrigerator, an air-conditioned laboratory, a humidified clean-room, and a humidified incubator respectively. Each time-treatment combination was replicated four times. The replicates were prepared, treated, and processed together. The data are summarized in Table 2.1.

The spores used in the experiment have the same genetic composition as the AAAC spores used in subsequent experiments but they are from an earlier crop whose production was less well-controlled. Therefore, the results are not directly comparable with the results of later experiments. The spores used in the present experiment were stored in alcohol for about a year before they were re-suspended in water in preparation for this experiment. The present results along with those of experiment 13 reported in Progress Report #1, suggest that spores produced under less than ideal conditions, and/or spores

which have been stored for a long time show a greater sensitivity to treatment humidity than do fresh, more-resistant spores such as those used in experiment DD9066; this possibility should be explored with additional species and varieties of spores.

Table 2.1 - D_{125}-values, Y-intercepts (y_o) and Their .95 Confidence Intervals and Intercept Ratios for Experiment DD8320. Comparison of the Effects of Four Treatment Humidities at One Conditioning Humidity

Pooled Data (4 replicates)

Conditioning RH at 23°C	Treatment RH at 125°C		Position 1	Position 5
40%	.36%	D y_o IR*	17.0 (12.4,26.7) 8.78E3 (8.00E2,9.65E4) .78	24.1 (17.8,37.2) 3.04E4 (5.92E3,1.56E5) .88
40%	.66%	D y_o IR	22.4 (16.3,36.1) 2.68E4 (4.05E3,1.77E5) .87	84.5 (24.7,∞) 1.26E4 (5.14E2,3.11E5) .81
40%	.79%	D y_o IR	20.7 (14.1,38.6) 1.73E4 (1.40E3,2.13E5) .83	31.5 (23.9,45.9) 2.75E4 (9.01E3,8.42E4) .87
40%	1.1%	D y_o IR	17.8 (12.9,28.7) 5.07E4 (4.69E3,5.48E5) .93	32.5 (25.5,44.9) 5.87E4 (2.27E4,1.52E5) .94

* $IR = \frac{\log y_o}{\log N_o}$ $\quad N_o$ = 1.20E5 (9.45E4,1.52E5)

The analysis of variance (Table 2.2) shows that treatment humidity has a significant effect on the thermal destruction of these spores in contrast to the results of experiment DD9066. As in all of the experiments, position has a significant effect with survival being lower near the edge of the mated surface plate than near the center. There is also evidence of interaction between humidity and position and also between treatment time and position; this means that the effect of treatment humidity and the effect of treatment time are probably dependent upon the location of the spores within the mated surface.

Table 2.2 - Analysis of Variance for Experiment DD8320: Comparison of the Effects of Four Treatment Humidities (.36,.66,.79,1.1% RH at 125°C) at One Conditioning Humidity (40% RH at 23°C)

Source	SS	df	MS	F
Mainplot				
Between 6" x 8" Sheets	214.8963	47	4.5723	
Humidity	40.7343	3	13.5781	14.97 (P<.001)
Treatment Time	132.3241	2	66.1621	72.94 (P<.001)
H x T	9.1819	6	1.5303	1.69 (not sig.)*
Mainplot Residual	32.6561	36	.9071	
Subplot				
Within 6" x 8" Sheets	104.6253	240	.4359	
Position	66.6957	5	13.3391	132.04 (P<.001)
Humidity x Position	3.3064	15	.2204	2.18 (P<.01)
Time x Position	12.6251	10	1.2625	12.50 (P<.001)
H x T x P	3.8142	30	.1271	1.26 (not sig.)*
Subplot Residual	18.1839	180	.1010	
Total	319.5217	287		

* P>.1

Data Analysis of Experiment DD9066

The experiment evaluated the effect of two levels of conditioning humidity (<2% and 90% RH at 23°C) and two levels of treatment humidity (.34% and 1.1% RH at 125°C) on thermal destruction. The experiment was carried out five times, each on different days. Each experiment used four treatment times (15,40,65, and 90 minutes) in addition to the various humidity treatments for a total of sixteen 6" x 8" plates or 96 1" x 2" strips per experiment, exclusive of controls. The preliminary results of the experiment were reported in Progress Report #2; the data are summarized in Table 2.3 and are shown in graphical form in Figure 2.2.

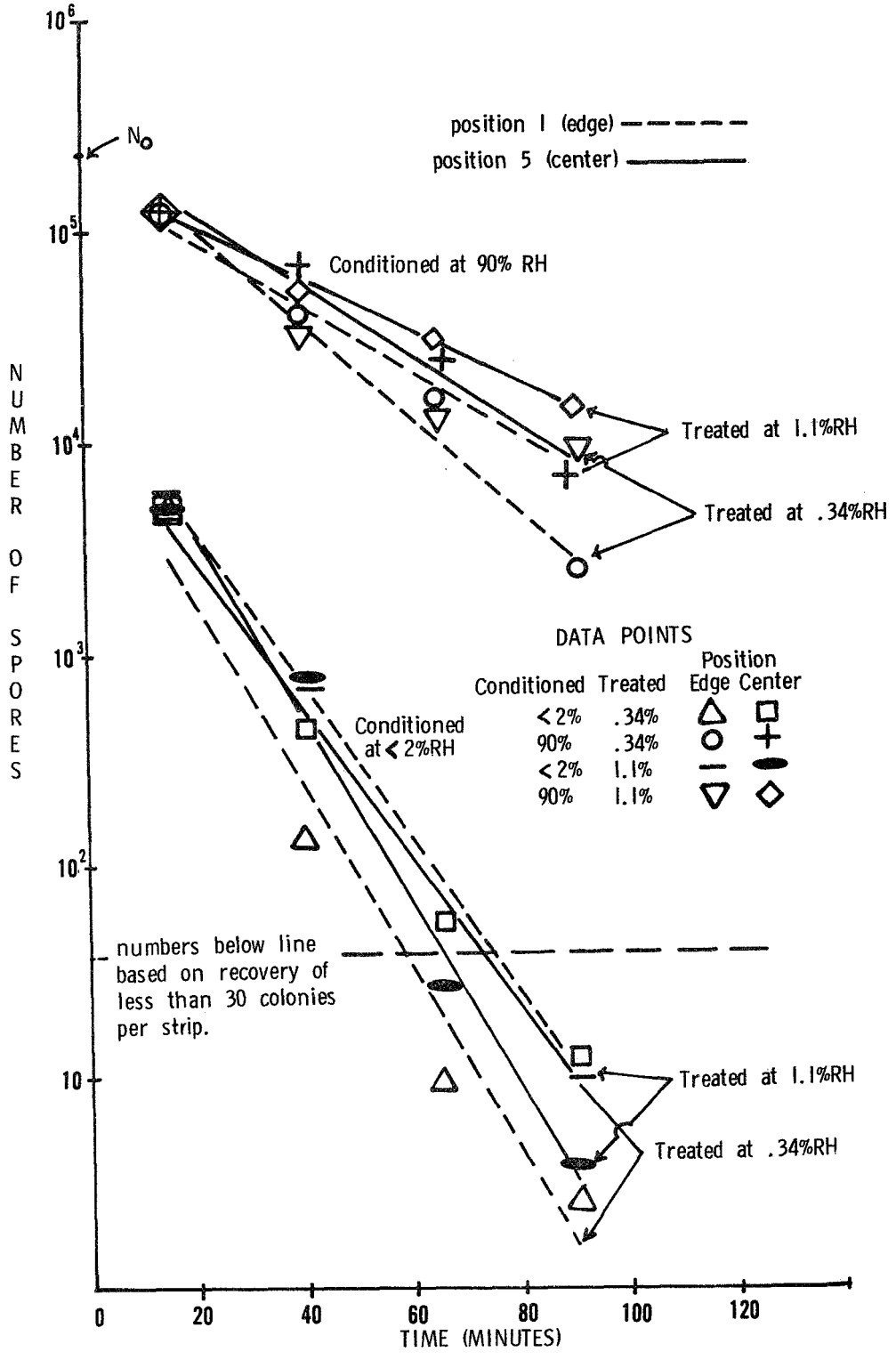

Figure 2.2 - Data points and least squares regression lines for Experiment DD9066: Comparison of the effects of two conditioning humidities (<2% and 90% RH at 23°C) and two treatment humidities (.34% and 1.1% RH at 125°C) in a mated surface system. Each datum point is the geometric mean recovery from five strips.

Table 2.3 - D_{125}-values, Y-intercepts (y_o) and Their .95 Confidence Intervals and Intercept Ratios for Experiment DD9066. Comparison of the Effects of Two Conditioning Humidities and Two Treatment Humidities in a Mated Surface System

Pooled Data(5 replicates)

Conditioning RH at 23°C	Treatment RH at 125°C		Position 1	Position 5
low <2%	low .34%	D	22.8 (16.2,38.7)	28.5 (18.0,69.3)
		y_o	1.28E4 (1.09E3,1.52E5)	1.33E4 (7.91E2,2.25E5)
		IR	.77	.77
low <2%	high 1.1%	D	27.2 (21.2,37.9)	23.0 (16.3,38.8)
		y_o	2.02E4 (4.87E3,8.37E4)	2.68E4 (2.37E3,3.04E5)
		IR	.81	.81
high 90%	low .34%	D	45.4 (37.4,57.9)	60.6 (44.5,94.9)
		y_o	2.81E5 (1.47E5,5.37E5)	2.49E5 (1.10E5,5.64E5)
		IR	1.02	1.01
high 90%	high 1.1%	D	65.6 (44.5,125.3)	82.5 (59.4,135.0)
		y_o	1.72E5 (6.38E4,4.65E5)	1.81E5 (9.47E4,3.44E5)
		IR	.98	.99

N_o = 2.13E5 (1.27E5,3.55E5)

If the results reported here are compared with those reported in Progress Report #2, a considerable difference in D-values will be noted; in the earlier report the arithmetic means were plotted for each time-treatment combination and a regression line was estimated visually. In the present experiment, the regression line was calculated using the logarithms of the data. The logarithm transformation makes the variance of the points independent of the mean, a necessary pre-condition for linear regression and analysis of variance. An equivalent visual procedure would be to plot the geometric means for each time-treatment combination and then estimate the regression line visually.

When spores are conditioned at <2% RH at 23°C and treated at .34% RH at 125°C, the D-value at position 1 is clearly less than the D-value at position 5. The results suggest that there is less water at position 1 than at position 5. When spores are conditioned at the lower humidity and treated at the higher humidity, however, position 1 at first appears to have a higher D-value than position 5; however, a look at the confidence intervals will show that the intervals overlap almost completely. We may say, and it is confirmed by the

analysis of variance, that the position effect is smaller at higher humidities; in other words, the difference in behavior between position 1 and position 5 is smaller at high treatment humidities than at low treatment humdities; probably because water vapor is lost from the exposed edge of the mated surface more slowly at higher treatment humidities.

When the conditioning humidity is high the results show a considerably larger D-value at the center position than at the edge position for both the low and high treatment conditions. This is compatible with the reasoning above since the surfaces conditioned at a higher humidity start the treatment cycle with a larger water content. Methods to measure the water content of a spore sample may soon be available; a direct measurement of water content combined with an analysis of the diffusion characteristics of the mated surface package should permit a relationship to be developed between water loss and thermal destruction. A revised mated system is proposed under "Future Work" which will make such an analysis more feasible.

The analysis of variance (Table 2.4) shows that the day on which an experiment is performed has a significant effect on the results. This result appears frequently in experiments with this system as well as with other systems; it requires further exploration. Conditioning humidity has a very significant effect, although treatment humidity does not. In the sub-plot analysis, however, a significant interaction between treatment humidity and position is detected. This is the variation in the effect of position with humidity which was discussed above. Position within the mated surface has a significant effect on the death parameters.

D-value data for the same spores (AAAC) at the same conditioning and treatment humidities but in an open system are reported as experiment DD9304.

Table 2.4 - Analysis of Variance for Experiment DD9066. Comparison of the Effects of Two Conditioning Humidities (<2% and 90% RH at 23°C) and Two Treatment Humidities (.34% and 1.1% RH at 125°C) in a Mated Surface System

Source	SS	df	MS	F
Mainplot				
Between 6" x 8" Sheets	1166.7464	79	14.7689	
Days	44.5468	4	11.1367	3.96 (P≐.09)
Conditioning Humidity	623.9927	1	623.9927	221.72 (P<.001)
Treatment Humidity	2.9505	1	2.9505	1.05 (not sig.)*
C x T	.3639	1	.3639	.13 (not sig.)*
Time	276.8597	3	92.2866	32.79 (P<.001)
Higher Interactions	49.1762	9	5.4640	1.94 (P≐.08)
Subjects within Groups	168.8566	60	2.8143	
Subplot				
Within 6" x 8" Sheets	44.6408	400	.1116	
Positions	2.0103	5	.4021	4.1 (P<.001)
Conditioning Humidity x Position	.3716	5	.0743	.76 (not sig.)*
Treatment Humidity x Position	2.6060	5	.5212	5.34 (P<.001)
Time x Position	1.3504	15	.0900	.92 (not sig.)*
Other Interactions with Position	7.0626	50	.1413	1.45 (P<.025)
Positions x Subjects within Groups	31.2399	320	.0976	
Total	1211.3873	479		

* P>.1

Experiment DD9141

The experiment repeated one of the large D-value conditions of experiment DD9066 using a treatment time sufficient to reduce the initial spore population by a factor of 1000; this helps to assure that no unusual behavior of the curve occurs after long treatment times. Another objective of the experiment was to investigate the day-to-day variability which occurs in this and other experimental systems. The question was: does

the variability appear among replicates handled on the same day or does it appear from day to day? In the former case, unsuspected variations in the treatment package or the experimental system would be suspected; in the latter case a variation in the preparation or processing or some other extraneous factor would be more likely.

The experiment was repeated on two different days. On each day two sets of mated surface plates were prepared, treated, and processed together. Three treatment times (1, 3.5, and 6 hours) were used. All test samples were conditioned at <2% RH at 23°C and were treated at .34% RH at 125°C. The results are summarized in Table 2.5.

Table 2.5 - D_{125}-values, Y-intercepts (y_o) and Their .95 Confidence Intervals and Intercept Ratios for Experiment DD9141. Up to Six-hour Treatment of Samples Conditioned at 90% RH at 23°C and Heated at .34% RH at 125°C

Day 1

	Position 1	Position 5
D	72.8 (56.6,101.9)	105.7 (65.4,275.5)
y_o	3.29E5 (3.67E4,2.95E6)	3.02E5 (1.15E4,7.92E6)
IR	1.05	1.04

Day 2

	Position 1	Position 5
D	64.1 (55.8,75.3)	72.3 (66.6,79.1)
y_o	3.37E5 (9.15E4,1.24E6)	5.28E5 (2.71E5,1.03E6)
IR	1.05	1.08

Pooled Data(2 replicates)

	Position 1	Position 5
D	68.2 (59.7,79.4)	85.9 (67.0,119.4)
y_o	3.33E5 (1.04E5,1.07E6)	3.99E5 (6.40E4,2.49E6)
IR	1.05	1.06

N_o = 1.90E5 (1.42E5,2.55E5)

The survivor curves behaved in the usual manner; however, the D-values obtained are somewhat longer than those for the same

conditions in experiment DD9066. The analysis of variance (Table 2.6) shows that the day-to-day variation is significant at the .05 level. The results confirm that it is better to carry out replicate experimental runs on different days rather than all on the same day. By carrying out the runs on different days, the mean D-values and intercepts will approach the real value more closely. The cause of the day-to-day variation is still unexplained; however, within days the inter-relationships of treatments are very well preserved, therefore the cause of the variation is probably external to the treatment system. Variations in barometric pressure or in some other atmospheric factor seem most likely, although variations in the recovery buffer or nutrient medium may be responsible. Further studies are most desirable to discover the source of the day-to-day variation.

Table 2.6 - Analysis of Variance for Experiment DD9141. Up to Six-hour Treatment of Samples Conditioned at 90% RH at 23°C and Heated at .34% RH at 125°C

Source	SS	df	MS	F
Mainplot				
Between 6" x 8" Sheets	217.6707	11	19.7882	
Days	7.9672	1	7.9672	6.42 (P<.05)
Treatment Time	199.5931	2	99.7965	78.97 (P<.001)
Mainplot Residual	10.1104	8	1.2638	
Subplot				
Within 6" x 8" Sheets	9.2740	60	.1546	
Position	3.9868	5	.7974	9.32 (P<.001)
Position x Process	1.4384	10	.1438	1.68 (not sig.)*
Subplot Residual	3.8488	45	.0855	
Total	226.9444	71	3.1964	

* P>.1

Experiment DD9155

The experiment compared the thermal destruction of spores deposited on type 301 stainless steel (rolled finish) with the destruction of spores

on type 302 stainless steel (brushed finish). Spores were conditioned at <2% RH at 23°C and were treated at .34% RH at 125°C. The experiment was completely replicated on two different days. The results are summarized in Table 2.7.

Table 2.7 - D_{125}-values, Y-intercepts (y_o) and Their .95 Confidence Intervals and Intercept Ratios for Experiment DD9155. Comparison of the Effects of Rolled Finish on Type 301 with Brushed Finish on Type 302 Stainless Steel Conditioned at <2% RH at 23°C and Heated at .34% RH at 125°C

		Position 1	Position 5
Type 301	D	52.4 (33.3,122.4)	77.6 (52.1,151.8)
	y_o	6.19E4 (1.39E4,2.76E5)	8.56E4 (3.61E4,2.03E5)
	IR	.91	.93
Type 302	D	106.6 (62.5,361.4)	82.9 (62.6,122.7)
	y_o	4.61E4 (1.86E4,1.14E5)	1.32E5 (7.68E4,2.25E5)
	IR	.88	.97

N_o = 1.94E5 (1.15E5,3.29E5)

Experiment 13 (reported in Progress Report #1) showed the major difference between the two surfaces with type 302 having a smaller D-value. This was attributed to the slightly rougher finish on the type 302 steel. However, the present experiment shows a reversal of the results of experiment 13 with the type 301 showing a smaller D-value in the present experiment. Although the difference between D-values was much smaller in the present experiment, the analysis of variance (Table 2.8) shows that the differences were significant at the .05 level.

The spores in experiment 13 were later found to be unreliable and were abandoned for experiment use; the large effect of surface finish in that experiment was probably due to the physico-chemical characteristics of the spores. The sensitivity of spores to humidity conditions seems to be increased by long storage and by poor spore preparation conditions. As mentioned above, a visual comparison of the results of experiments DD8320 and DD9066 suggests that, especially during the short period when the spores are in the treatment environment, newer and more resistant spores are less likely to show differences in death parameters, due to differences in the treatment system. Further investigation of the effects

of surface finish on various types of spores may be desirable.

Table 2.8 - Analysis of Variance for Experiment DD9155. Comparison of the Effects of Rolled Finish on Type 301 with Brushed Finish on Type 302 Stainless Steel Conditioned at <2% RH at 23°C and Heated at .34% RH at 125°C

Source	SS	df	MS	F
Mainplot				
Between 6" x 8" Sheets	19.5467	15	1.3031	
Days	.0248	1	.0248	.10 (not sig.)*
Surface Finish	1.6050	1	1.6050	6.43 (P<.05)
Treatment Time	15.3837	3	5.1279	20.55 (P<.001)
S x T	.7865	3	.2622	1.05 (not sig.)*
Mainplot Residual	1.7467	7	.2495	
Subplot				
Within 6" x 8" Sheets	4.7085	80	.0589	
Positions	3.1653	5	.6331	35.11 (P<.001)
Position x Process	.8222	35	.0235	1.30 (not sig.)*
Subplot Residual	.7211	40	.0180	
Total	24.2552	95	.2553	

* P>.1

Experiment DD9161

The experiment compared the thermal destruction of spores in the usual mated surface package, with the destruction in a package in which a longitudinal .010 inch separator was placed between the plates. The shape of the separator used is shown in Figure 4.2 of Progress Report #1. The spores were conditioned at <2% RH at 23°C and were treated at .34% RH at 125°C. The experiment was replicated on two different days. The results are shown in Table 2.9.

Table 2.9 - D_{125}-values, Y-intercepts (y_o) and Their .95 Confidence Intervals and Intercept Ratios for Experiment DD9161. Comparison of the Effects of Packages With and Without a .010 inch Separator Between the Mated Surfaces

Pooled Data(2 replicates)

		Position 1	Position 2
Without Separator	D	36.4 (24.1,73.5)	43.3 (28.2,93.0)
	y_o	1.61E5 (2.31E4,1.13E6)	1.77E5 (3.15E5,9.96E5)
	IR	.99	1.00
With .010 inch Separator	D	40.0 (36.0,45.0)	48.6 (38.5,65.7)
	y_o	1.01E5 (6.87E4,1.49E5)	1.08E5 (5.08E4,2.29E5)
	IR	.95	.96

N_o = 1.76E5 (1.28E5,2.42E5)

The analysis of variance (Table 2.10) shows that the presence or absence of a separator has no significant effect on spore destruction.

Table 2.10 - Analysis of Variance for Experiment DD9161. Comparison of the Effects of Packages With and Without .010 inch Separator Between the Mated Surfaces. Conditioned at <2% RH at 23°C and Heated at .34% RH at 125°C

Source	SS	df	MS	F
Mainplot				
Between 6" x 8" Sheets	39.4088	11	3.5826	
Days	.0975	1	.0975	.22 (not sig.)*
Separator Thickness	.1944	1	.1944	.43 (not sig.)*
Treatment Time	36.8231	2	18.4115	41.16 (P<.001)
S x T	.0575	2	.0288	.06 (not sig.)*
Mainplot Residual	2.2363	5	.4473	
Subplot				
Within 6" x 8" Sheets	2.1648	60	.0361	
Positions	.8632	5	.1726	7.51 (P<.001)
Position x Process	.6117	25	.0245	1.06 (not sig.)*
Subplot Residual	.6899	30	.0230	
Total	41.5736	71		

* P>.1

Experiment DD9169

The experiment was carried out using spores suspended in water and having no history of alcohol storage. It had been suspected that water-stored spores behaved differently from spores with a history of alcohol storage and it was hoped that the results of this survey experiment might provide additional information with which to interpret the results of previous experiments. Three types of treatment packages were used: the ordinary package prepared with type 301 (rolled surface) stainless steel, the package with .010 inch separators as used in experiment DD9161 and the ordinary package prepared with type 302 (brushed surface) stainless steel. The test samples were conditioned at 40% RH and treated at .34% RH at 125°C. The results are shown in Table 2.11. Only four samples per plate were recovered and the experiment was carried out on only one day since only relative values were desired and the experiment was performed as a survey.

Table 2.11 - D_{125}-values, Y-intercepts (y_o) and Their .95 Confidence Intervals and Intercept Ratios for Experiment DD9169. Effects of Three Types of Mated Surfaces on Spores (AABF) Conditioned at 40% RH at 23°C and Heated at .34% RH at 125°C

		Position 1	Position 5
301 Surface Without Separator (standard package)	D y_o IR	29.8 (8.7,∞) 2.43E5 (1.75E2,3.38E8) .98	36.5 (4.5,∞) 2.95 (9.35E-3,9.32E12) 1.00
301 Surface .010-inch Separator	D y_o IR	25.3 (3.0,∞) 2.26E5 (1.28E-6,3.99E16) .98	30.3 (10.1,∞) 3.46E5 (9.65E2,1.24E8) 1.01
302 Surface Without Separator	D y_o IR	42.5 (22.4,414.0) 1.96E5 (3.00E4,1.28E6) .97	62.4 (14.8,∞) 2.65E5 (2.72E3,2.55E7) .99

N_o = 2.94E5 (2.46E5,3.51E5)

The presence or absence of a separator seems to have no effect. The type 302 surface seems to produce an increase in D-value which might be found to be significant if replicate experiments were carried out. The results are similar to the results of experiments DD9155 and DD9161 which

measured the behavior of spores with a history of alcohol storage.

Experiment DD9176

The spores in this experiment were conditioned at <2% RH at 23°C and treated at .46% RH at 115°C. The results are shown in Table 2.12.

Table 2.12 - D_{115}-values, Y-intercepts (y_o) and Their .95 Confidence Intervals and Intercept Ratios for Experiment DD9176. Conditioned at <2% RH at 23°C and Heated at .46% RH at 115°C

	Position 1	Position 5
D	211.8 (205.7,218.3)	234.6 (63.2,∞)
y_o	1.05E5 (9.75E4,1.14E5)	1.73E5 (2.66E2,1.12E8)
IR	.95	.99

N_o = 1.97E5 (1.62E5,2.39E5)

The results represent only one mated surface package per time-treatment combination. If the average D-value for the six recovered positions on the plate (292 minutes) is compared with the weighted average of the portions of experiments DD9155,DD9161 and DD9066 which were similarly treated, a Z-value of 11°C is obtained. This value is smaller than most of the dry heat Z-values reported in the literature; it is important that we determine whether this is an artifact or is characteristic of the mated surface system. The D-value confidence interval for the pooled data of experiment DD9176 is 218 minutes to 440 minutes. Using these extremes gives a range for the Z-value of 9-13 minutes which is roughly analogous to a .95 confidence interval. It should be noted that the treatment humidity for experiment DD9176 is slightly higher than that of the experiments treated at 125°C due to limited control on the treatment environment used.

Further study at several humidity conditions and treatment temperatures using the same treatment humidity at each temperature will be needed.

Experiment DD9273

The experiment evaluated the effect of three conditioning humidities. Samples were conditioned at <2%, 36% and 90% relative humidities at 23°C and were treated at .34% RH at 125°C. The experiment was replicated on two different days. The data are summarized in Table 2.13.

Table 2.13 - D_{125}-values, Y-intercepts(y_o) and Their .95 Confidence Intervals and Intercept Ratios for Experiment DD9273. Comparison of the Effects of Three Conditioning Humidities at One Treatment Humidity (.34% RH at 125°C)

Pooled Data (2 replicates)

Conditioning RH at 23°C		Position 1	Position 5
low <2%	D	20.8 (12.7,57.2)	33.1 (20.7,84.0)
	y_o	3.44E4 (9.33E2,1.27E6)	4.92E4 (5.71E3,4.25E5)
	IR	.88	.91
medium 36%	D	38.1 (27.5,61.8)	38.9 (30.0,55.6)
	y_o	4.32E4 (1.32E4,1.42E5)	1.26E5 (5.09E4,3.12E5)
	IR	.90	.99
high 90%	D	68.4 (54.7,91.3)	145.6 (53.0,)
	y_o	2.22E5 (1.44E5,3.43E5)	1.26E5 (3.06E4,5.20E5)
	IR	1.04	.99

N_o = 1.41E5 (6.64,3.00E5)

As has been shown in previous mated surface experiments, conditioning humidity has a large effect on D-value. This is confirmed in the analysis of variance in Table 2.14. The results show again that the y-intercept of the survivor curve as well as its slope is affected by the conditioning humidity.

Table 2.14 - Analysis of Variance for Experiment DD9273. Comparison of the Effects of Three Conditioning Humidities (<2%, 36% and 90% RH at 23°C) at One Treatment Humidity (.34% RH at 125°C)

Source	SS	df	MS	F
Mainplot				
Between 6" x 8" Sheets	95.4911	17	5.6171	
Days	.5797	1	.5797	2.37 (not sig.)*
Humidity	56.3923	2	28.1962	115.51 (P<.001)
Treatment Time	30.9255	2	15.4628	63.35 (P<.001)
H x T	5.6412	4	1.4103	5.78 (P<.005)
Mainplot Residual	1.9524	8	.2441	
Subplot				
Within 6" x 8" Sheets	18.2419	90	.2027	
Positions	9.5284	5	1.9057	28.07 (P<.001)
Humidity x Position	2.5324	10	.2532	3.73 (P<.005)
Time x Position	1.5505	10	.1551	2.28 (P<.05)
H x T x P	1.5745	20	.0787	1.16 (not sig.)*
Subplot Residual	3.0561	45		
Total	113.7331	107	.0679	

* P>.1

The extreme conditioning humidities of this experiment and the conditioning humidities of experiment DD9066 are the same so the results may be compared if the confounding factor of day-to-day variation is kept in mind. The data are compared in Table 2.15.

Table 2.15 - D-values, Y-intercepts(y_o) and Intercept Ratios for Comparable Conditions from Experiments DD9066 and DD9273 (treated at .34% RH at 125°C)

		Position 1		Position 5	
		DD9066	DD9273	DD9066	DD9273
Conditioned at <2% RH at 23°C	D	22.8 (16.2,38.7)	20.8 (12.7,57.2)	28.5 (18.0,69.3)	33.1 (20.7,84.0)
	y_o	1.28E4 (1.09E3,1.52E5)	3.44E4 (9.33E2,1.27E6)	1.33E4 (7.91E2,2.25E5)	4.92E4 (5.71E3,4.25E5)
	IR	.77	.88	.77	.91
Conditioned at 90% RH at 23°C	D	45.4 (37.4,57.9)	68.4 (54.7,91.3)	60.6 (44.5,94.9)	145.6 (53.0,∞)
	y_o	2.81E5 (1.47E5,5.37E5)	2.22E5 (1.44E5,3.43E5)	2.49E5 (1.10E5,5.64E5)	1.26E5 (3.06E4,5.20E5)
	IR	1.02	1.04	1.01	.99

The D-values for the low humidity tests were of the same magnitude in both experiments and in general the D-value at position 1 did not differ greatly from the D-value at position 5 although in both cases the D-values at position 5 were larger. When the spores were conditioned at 90% RH at 23°C, considerably higher D-values were obtained; the high humidity D-values in experiment DD9273 were from 50% to more than 150% larger than the D-values of experiment DD9066. In both cases the D-values at position 5 were also 50-150% greater than the D-values at position 1. The significance of these results is not understood at this time.

Experiment DD9277-open system

The experiment was carried out to provide data on the dry heat destruction of spores in an open system at an intermediate conditioning and treatment humidity. Spores were conditioned at 36% RH at 23°C and were treated at .44% RH at 125°C. Two sets of five samples were prepared, treated, and processed for each time-treatment combination. The experiment was carried out on only one day. The treatment system is described in Progress Report #2. The results are shown in Table 2.16.

Table 2.16 - D_{125}-values, Y-intercepts(y_o) and Their .95 Confidence Intervals and Intercept Ratios for Experiment DD9277. Conditioned at 36% RH at 23°C and Heated at .44% RH at 125°C in an Open System

Replicate 1	D y_o IR	16.2 (13.2,21.0) 1.56E4 (3.49E3,6.96E4) .81
Replicate 2	D y_o IR	18.8 (13.8,29.4) 9.26E3 (1.22E3,7.03E4) .77
Pooled	D y_o IRF	17.4 (14.5,21.6) 1.20E4 (3.64E3,3.97E4) .78

N_o = 1.47E5 (1.26E5,1.71E5)

Experiment DD9304-open system

The experiment is similar to the open-system experiment reported in Progress Report #2. Samples were conditioned at less than 2% RH or at 89% RH at 23°C and were treated at .34% or 1.1% RH at 125°C. The conditions are comparable to those of experiment DD9066 for a mated system. The experiment was completely replicated on two different days. In each experiment, three 1" x 2" strips, each carrying one spore deposit were assigned to each time-treatment combination. This gives a total of six replicate samples for each time-treatment combination.

In the earlier experiment there was an unavoidable delay between removal of the strips from the conditioning environment and their insertion into the treatment environment. In the present experiment, the time was

reduced from four minutes to 20 seconds. The temperature of each strip was monitored throughout treatment and the final counts were corrected to a treatment temperature of 125°C using a Z-value of 21°C. The results are shown numerically in Table 2.17 and graphically in Figure 2.3.

Table 2.17 - D_{125}-values, Y-intercepts(y_o) and Their .95 Confidence Intervals and Intercept Ratios for Experiment DD9304. Comparison of the Effects of Two Conditioning Humidities and Two Treatment Humidities in an Open System

Conditioning RH at 23°C	Treatment RH at 125°C		Day 1(3 strips)	Day 2(3 strips)	Pooled(6 strips)
low 2%	low .34%	D	13.7 (11.1,18.1)	19.3 (16.0,24.3)	16.1 (13.1,20.1)
		y_o	6.13E4 (9.79E3,3.85E5)	7.85E4 (2.56E4,2.41E5)	6.94E4) (1.62E4,2.97E5)
		IR	.89	.91	.90
low 2%	high 1.1%	D	17.5 (14.2,22.6)	23.9 (21.9,26.4)	20.6 (18.5,23.1)
		y_o	4.83E5 (1.23E5,1.90E6)	3.06E5 (2.03E5,4.60E5)	3.78E5 (2.13E5,6.69E5)
		IR	1.06	1.02	1.04
high 89%	low .34%	D	15.8 (13.2,19.8)	22.0 (19.0,26.2)	18.4 (14.7,24.5)
		y_o	1.22E5 (3.20E4,4.63E5)	3.29E5 (1.53,7.07E5)	2.00E5 (4.79E4,8.36E5)
		IR	.95	1.03	.99
high 89%	high 1.1%	D	32.5 (24.2,49.8)	44.5 (34.7,62.1)	37.6 (29.5,51.9)
		y_o	1.62E5 (5.28E4,5.00E5)	2.16E5 (1.10E5,4.22E5)	1.87E5 (8.65E4,4.05E5)
		IR	.97	.99	.98

N_o = 2.34E5 (1.74E5,3.15E5)

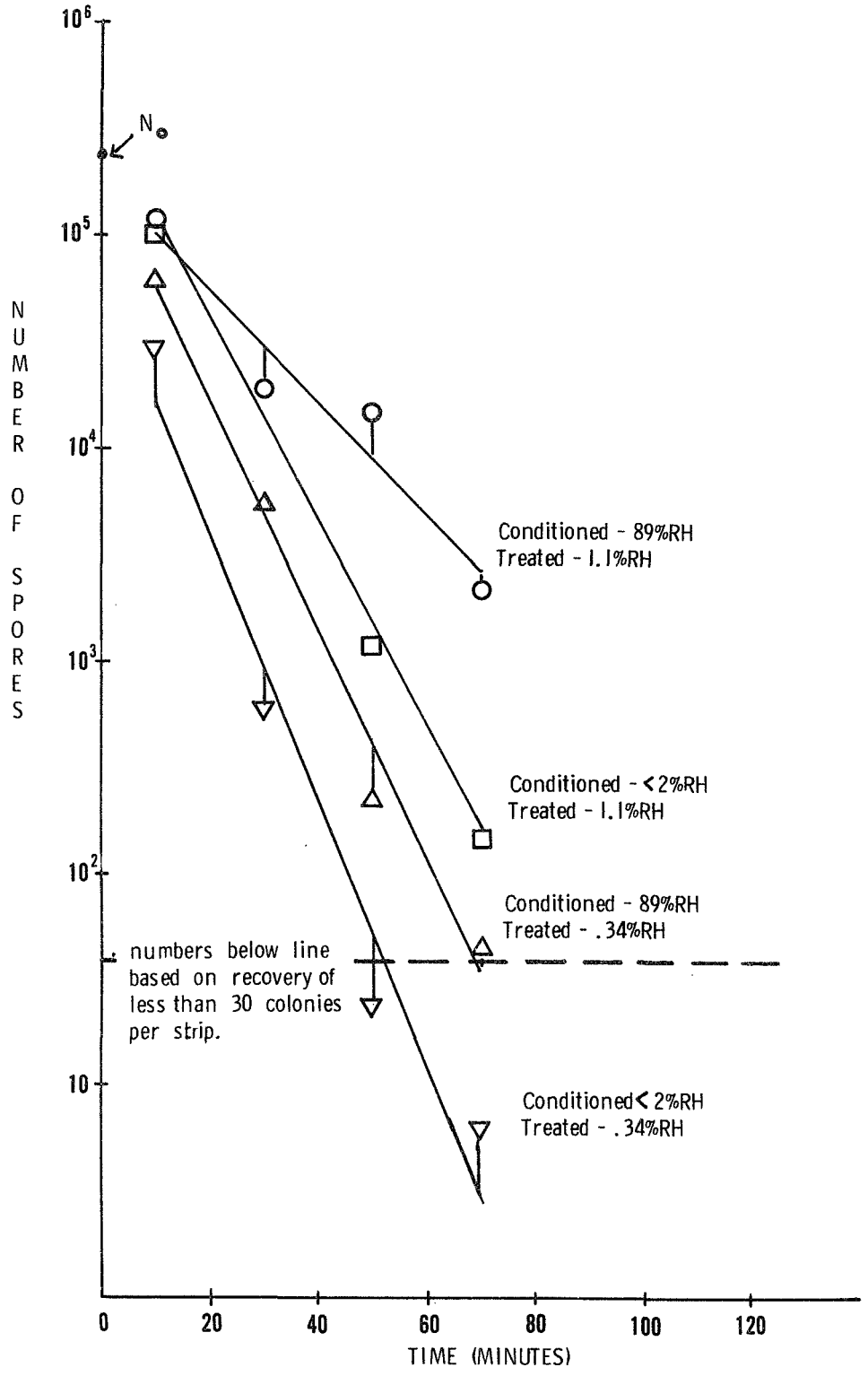

Figure 2.3 - Data points and least squares regression lines for Experiment DD9304: Comparison of the effects of two conditioning humidities (<2% and 90% RH at 23°C) and two treatment humidities (.34% and 1.1% RH at 125°C) in an open system. Each datum point is the geometric mean recovery from six strips.

The analysis of variance (Table 2.18) shows that in the open system, both conditioning and treatment humidity have an effect on the thermal destruction of spores. In the mated system, it will be remembered, conditioning humidity is clearly the most important factor. The analysis of variance also shows that position did not have a significant effect.

A comparison of Figure 2.2 (mated system, page 20) and Figure 2.3 (open system) shows the relatively larger effect of treatment RH in the open system.

Table 2.18 - Analysis of Variance for Experiment DD9304. Comparison of the Effects of Two Conditioning Humidities(<2% and 90% RH at 23°C) and Two Treatment Humidities(.34% and 1.1% RH at 125°C) in an Open System

Source	SS	df	MS	F
Mainplot				
Between Racks of 3 Strips	185.9220	31	5.9975	
Days	12.6575	1	12.6575	30.48 (P<.001)
Conditioning RH	11.0480	1	11.0480	26.60 (P<.001)
Treatment RH	33.4900	1	33.4900	80.64 (P<.001)
C x T	.2465	1	.2465	.59 (not sig.)*
Treatment Time	111.1031	3	37.0344	89.18 (P<.001)
Other Interactions	11.1478	9	1.2386	2.98 (P<.05)
Mainplot Residual	6.2291	15	.4153	
Subplot				
Within Racks of 3 Strips	5.8389	64	.0898	
Position	.1632	2	.0816	.82 (not sig.)*
Position x Process	2.4000	30	.0800	.81 (not sig.)*
Subplot Residual	3.2757	32	.0993	
Total	191.7607	95	2.0185	2.0185

* P>.1

Experiment DD9329

The experiment was a comparison between the AAAC spores which have been used in nearly all the mated surface experiments, the same spores diluted tenfold, and VDLA spores (Mariner Isolate #4-47A grown on synthetic sporulation medium) supplied by Jet Propulsion Laboratory. The two different dilutions of the AAAC spores were used to see whether deposition from a more dilute suspension, which theoretically would decrease clumping, would have an effect on thermal destruction. The spores were conditioned at 36% RH at 23°C and were treated at .34% RH at 125°C. The results are shown in Table 2.19.

Table 2.19 - D_{125}-values, Y-intercepts(y_o) and Their .95 Confidence Intervals and Intercept Ratios for Experiment DD9329. Comparison of AAAC Spores with VDLA Spores Conditionedaat 36% RH at 23°C and Heated at .34% RH at 125°C

		Position 1	Position 5
AAAC N_o=1.92E5	D	29.3 (23.2,39.5)	42.9 (36.5,51.9)
	y_o	2.15E5 (5.30E4,8.76E5)	1.81E5 (9.55E4,3.45E5)
	IR	1.01	.99
AAAC N_o=1.92E4	D	31.7 (10.0,∞)	72.3 (66.9,78.7)
	y_o	1.59E4 (5.34E0,4.76E7)	9.82E3 (8.60E3,1.12E4)
	IR	.98	.93
VDLA N_o=1.92E5	D	159.8 (48.5,∞)	131.4 (70.5,966.0)
	y_o	1.45E5 (1.49E4,1.41E6)	2.70E5 (9.53E4,7.63E5)
	IR	.98	1.03

Up to the 105-minute maximum treatment time, the VDLA spores showed much greater heat resistance than did the AAAC spores. A final judgment on the resistance of the VDLA isolate will require repeated experiments using longer treatment times. The treatment time should be long enough for the survivor curve to traverse at least three log cycles. This is especially true in this case since some workers have reported a decreasing D-value with increasing treatment times for this and related spores. The results of the comparison between the usual concentration of AAAC in these tests (2×10^5 per .02 ml) and the

diluted concentration (2×10^4 per .02 ml) of the AAAC spores is also inconclusive at this time.

CONCLUSIONS

1. Treatment humidity has a much larger effect on D-values in an open system than it does in a mated-surface system. The treatment humidity has an effect in mated systems, however, it may be neglible as far as the design of spacecraft sterilization cycles are concerned.
2. In the open system used here, both conditioning and treatment humidity affect D-value, y-intercept and IR.
3. Both the D-value and y-intercept must be considered in a discussion of microbial survival. The intercept ratio (IR) appears to be useful for discussion.
4. The mated-surface system shows a greater variability than does the open system. This is not unexpected and is probably due to variabilities in the mated-surface package and in the off-the-shelf stainless steel used for the mated surfaces. It emphasizes, however, the need for replicating data where high precision is desired.
5. The location of spores in a mated surface has an important effect on their thermal destruction rate. The spores near the edge tend to have smaller D-values than spores at the center of the 6" x 8" plate.

FUTURE WORK

The measurement of Z-values and of D, y_o, and IR in dry nitrogen will complete phase I of the mated-surface study. These are the last experiments contemplated for the present heat block.

For phase 2 of the study, a new disc-shaped treatment apparatus will be constructed. A disc-shaped mated surface without packaging material would lend itself to engineering analysis more readily than would the rectangular mated surfaces used in the phase I study. The apparatus will be constructed in several sizes to simulate different

sizes of mated surfaces. In addition, a number of units of each size will be constructed so that multiple samples can be treated simultaneously; this will be particularly important as we attempt to measure the survival of very small numbers of organisms.

Several surface materials, both metallic and plastic, will be used to simulate materials present on the spacecraft. The effect of storage time in dry nitrogen after assembly and conditioning under clean room conditions and before treatment in dry nitrogen should be explored, since mated surfaces re-equilibrate to new humidity conditions more slowly than do open surfaces.